

Running Title: Influence of Extracellular Matrix on the Nucleus

Title: Extracellular Matrix, Nuclear and Chromatin Structure and Gene Expression in Normal Tissues and Malignant Tumors: A Work in Progress

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Abstract

Almost three decades ago, we presented a model where the extracellular matrix (ECM) was postulated to influence gene expression and tissue-specificity through the action of ECM receptors and the cytoskeleton. This hypothesis implied that ECM molecules could signal to the nucleus and that the unit of function in higher organisms was not the cell alone, but the cell plus its microenvironment. We now know that ECM invokes changes in tissue and organ architecture and that tissue, cell, nuclear and chromatin structure are changed profoundly as a result of and during malignant progression. Whereas some evidence has been generated for a link between ECM-induced alterations in tissue architecture and changes in both nuclear and chromatin organization, the manner by which these changes actively induce or repress gene expression in normal and malignant cells is a topic in need of further attention. Here, we will discuss some key findings that may provide insights into mechanisms through which ECM could influence gene transcription and how tumor cells acquire the ability to overcome these levels of control.

I. Introduction

“No man is an island, entire of itself; every man is a piece of the continent, a part of the main.”

John Donne (1573-1631)

Over the years, studies of transcription, chromatin structure and nuclear organization, mostly performed in lower organisms or in cells isolated from higher organisms and cultivated on tissue culture plastic, have provided important and valuable evidence for elucidating intracellular signaling events important for gene expression. But much still remains to be elucidated in order to understand the intricate complexity of gene expression within a three-dimensional tissue context. Vertebrate cells exist as part of an integrated unit of multiple cell types, i.e. an organ, in a milieu rich in growth factors, hormones and an assortment of ECM molecules, the combination of which is organ-specific. Similarly, the nucleus within each cell is not an isolated vessel. It is connected to its external milieu and the ECM through a dynamic intracellular network that includes the nuclear matrix, the nuclear envelope and the cytoskeleton. In the past few decades, a number of studies have provided evidence that the physical and biochemical signals transmitted from the ECM to the nucleus are indeed dynamic and reciprocal (Bissell et al., 1982; Lelievre et al., 1998; Plachot and Lelievre, 2004; Roskelley et al., 1994; Zoubiane et al., 2004). More specifically, the nature of ECM receptors and their signaling, and the nature of the connections between these receptors and intracellular components, such as the cytoskeleton and its interacting proteins, promote changes in nuclear events which influence gene expression and initiate the production of specific signaling molecules that are fed back into the extracellular environment which, in turn, influences the cell and the nucleus (Bissell et al., 1982) (Figure 1). Understanding how signaling by the ECM and its receptors are integrated into messages that alter chromatin structure and nuclear function is essential for knowing how a normal cell

functions in the context of a tissue and an organ, and for identifying aberrations that lead to the development of diseases such as cancer. Here we will discuss the influence of ECM on cellular and nuclear functions, as well as chromatin structure, and relate these changes to alterations in transcriptional events essential for functional differentiation and transformation.

II. The Extracellular Matrix

There are two broad subtypes of ECM: interstitial/stromal ECM and basement membrane (BM) (Guo and Giancotti, 2004). The interstitial matrix surrounds cells in the connective tissue (Guo and Giancotti, 2004), while the BM is present at the basolateral surface of different cell types in many tissues (Kalluri, 2003). The BM is composed mainly of laminin, type IV collagen, entactin/nidogen, and proteoglycans such as heparin sulfate that are deposited by many different cell types (Kalluri, 2003).

In order to fully appreciate how profoundly a cell and its nucleus are affected by the ECM, one must consider that the composition of the ECM is constantly influenced by physiological effectors such as growth factors, cytokines, and hormones, and, thus, is continuously changing throughout development, ageing, tissue repair and tumor progression (Guo and Giancotti, 2004; Labat-Robert, 2003; Mott and Werb, 2004). There are now multiple types of ECM receptors that have been identified including integrins, syndecans, glypicans and dystroglycan (Guo and Giancotti, 2004; Rizki and Bissell, 2004; Weir and Muschler, 2003). Different integrins are known to activate distinct signaling pathways but the same integrin receptor can stimulate multiple signaling pathways (DeMali et al., 2003; Guo and Giancotti, 2004).

Exposure to ECM promotes changes in cell shape, initiates the clustering of neighboring integrin receptors at cell-matrix contact sites, and promotes the recruitment of kinases and adaptor

proteins that link these receptor molecules to the actin cytoskeleton (Schatzmann et al., 2003). These changes initiate a series of mechanical and biochemical signals within the cell that ultimately change the program of gene expression and influence cellular processes such as survival, transcription factor activation, and apoptosis (Schatzmann et al., 2003). In the case of mammary epithelial cells, changes in cell shape that are mediated by exposure to a laminin-rich ECM gel (lrECM) activate expression of the milk protein lactoferrin (Close et al., 1997; Roskelley et al., 1994) (Figure 2). When these cell shape changes are combined with further biochemical and mechanical signals generated from the prolactin receptor and laminin 1-induced activation of integrin receptors, the transcription of additional milk proteins such as β -casein is initiated, but the expression of growth factors such as TGF- β 1 is inhibited concomitantly (Streuli et al., 1991; Streuli et al., 1995; Streuli et al., 1993). Following initial exposure to lrECM, morphogenic events take place that lead to the development of spherical, acinar structures with hollow lumen (Barcellos-Hoff et al., 1989). The formation of these structures is accompanied by growth arrest, enhancement of milk protein production, down-regulation of growth factors such as TGF- α , and the expression of new milk proteins such as whey acidic protein (WAP) (Barcellos-Hoff et al., 1989; Chen and Bissell, 1989; Lin et al., 1995; Petersen et al., 1992). As a result, the ability of lrECM to promote the functional differentiation of normal mammary epithelial cells into polar, acinar structures that produce and secrete milk requires several levels of control (Bissell et al., 1999; Lin and Bissell, 1993; Roskelley et al., 1995).

III. ECM-Response DNA Elements

One of the most convincing pieces of evidence linking the ECM to gene expression was the identification of an ECM response element in the promoter region of the bovine β -casein gene. Deletion analysis of the bovine β -casein promoter revealed that the transcriptional activation of

this promoter was completely dependent on a 160 bp BCE-1 transcriptional enhancer located approximately 1.5 kb upstream of the transcription start site (Schmidhauser et al., 1992). Since this discovery, ECM response elements have been identified in several other genes from different species (DiPersio et al., 1991; Novaro et al., 2004; Schmidhauser et al., 1994; Schmidhauser et al., 1992) (See Table 1). However, exactly how the ECM influences gene expression through these response elements is poorly understood.

IV. Potential Mechanisms for the Transcriptional Activation of ECM-Response DNA Elements

A). Exposure to ECM influences the nuclear translocation and DNA binding properties of specific transcription factors that bind to ECM-response elements.

One mechanism through which exposure to ECM may influence the activation of response elements is by altering the levels of specific transcription factors residing in the nucleus.

Cognate DNA binding elements for STAT5, and C/EBP β reside in both the bovine β -casein and mouse ER α ECM-response elements (Myers et al., 1998; Novaro et al., 2004). Mutation analysis of these binding sites revealed that their requirement for activation of the β -casein promoter is absolute (Myers et al., 1998). In mammary epithelial cells, exposure to lrECM and prolactin promotes the phosphorylation and translocation of STAT5 into the nucleus (Edwards et al., 1998; Gouilleux et al., 1994; Groner and Gouilleux, 1995; Ihle, 1996). Chromatin-binding proteins locate their cognate sites by scanning the 3D space of the nucleus and bind transiently to chromatin with a residence time on the order of seconds (Phair et al., 2004). An increase in STAT5 levels within the nucleus likely increases the frequency with which this factor interacts with its target DNA sites. In support of this, we have recently observed that exposure of mouse

mammary epithelial cells to IrECM and prolactin induces the recruitment of STAT5 to the β -casein promoter (Xu et al., 2005). Whether IrECM treatment alters the nuclear levels of other factors important for β -casein expression such as C/EBP β or GR remains to be determined. In a previous study, the culturing of primary rabbit mammary epithelial cells on collagen increased the expression levels of C/EBP β (Jolivet et al., 2001). In addition, we have also observed an increase in the binding of C/EBP β to the endogenous mouse β -casein promoter in response to IrECM and prolactin treatment (Xu et al., 2005). Such evidence supports the possibility that IrECM affects gene transcription by influencing transcription factors levels within the nucleus.

B). Exposure to ECM may initiate mechanical signals that alter the organization of nuclear factors in a manner that promotes activation of ECM-response elements

However, the possibility remains that the expression level of some nuclear proteins does not change in response to ECM treatment. Exposure to ECM has profound effects on cell shape, and cytoskeletal organization (Roskelley et al., 1994; Zoubiane et al., 2004) and the integrity of the cytoskeleton is essential for both STAT5 nuclear translocation and β -casein transcription (Phung-Koskas et al., 2005; Roskelley et al., 1994; Zoubiane et al., 2004). Furthermore, the cytoskeleton bridges the nucleus with the ECM by interacting with both nuclear envelope proteins and integrin receptors on the plasma membrane (Crisp et al., 2006; D'Angelo and Hetzer, 2006; Fey et al., 1984a; Fey et al., 1984b; Janmey, 1998; Wilhelmsen et al., 2005). Lamins help constitute the nuclear envelope and organize DNA into loop domains (Davie, 1995). Alterations in the organization of cytoskeletal filaments, distortions in nuclear shape and a redistribution of nucleoli along an axis of applied tension have been observed in bovine capillary epithelial cells in response to the mechanical tug of an integrin receptor lining the cell surface

(Maniotis et al., 1997; Maniotis et al., 2005). Thus, it is possible that the ECM promotes transcriptional activation of response elements by changing cytoskeletal organization, which may, in turn, alter the arrangement of nuclear components to promote a localized concentration of transcription factors to areas of the nucleus containing target DNA sequences. In addition, exposure to ECM may also stimulate cellular events that post-translationally modify and, therefore, alter the properties of transcription factors and chromatin remodeling enzymes.

C). ECM-induced activation of DNA response elements involves mechanisms that invoke changes in chromatin structure

Regardless of whether the ECM mediates an increase in the nuclear levels of transcription factors and/or an increase in the proximity of these factors to their target DNA binding sites, the fact remains that exposure of mammary epithelial cells to lrECM induces STAT5 and C/EBP β binding to DNA sites that are within close proximity of one another within the BCE-1 element and the mouse β -casein promoter (Myers et al., 1998; Rosen et al., 1999; Xu et al., 2005). We have shown previously that the treatment of stably transfected mouse mammary epithelial cells with a general histone deacetylase inhibitor increases BCE-1 activity, albeit not to the same extent as cells treated with inhibitor and lrECM together (Myers et al., 1998). In a recent study, we also observed an increase in the association of acetylated histones with the endogenous mouse β -casein promoter after lrECM and prolactin treatment (Xu et al., 2005). Further examination of the effects of lrECM on transcription factor recruitment revealed that exposure to lrECM induced also the binding of Brg1 (Xu et al., 2005), an ATPase subunit of the SWI/SNF chromatin remodeling complex (de la Serna et al., 2006). Over-expression of a dominant negative Brg1 isoform significantly decreased β -casein RNA levels (Xu et al., 2005). These

results indicate that IrECM-mediated induction of β -casein gene transcription requires both histone acetylation and ATP-dependent chromatin remodeling.

STAT5, GR and C/EBP β have been shown to interact with histone acetyltransferases (Lee et al., 1985; Li et al., 2003; Litterst et al., 2003; Pfitzner et al., 1998) and the transactivation potential of GR depends on its ability to recruit the chromatin remodeling factor Brg1 to target genes (Nagaich et al., 2004; Trotter and Archer, 2004). We have observed by co-immunoprecipitation analyses that STAT5, C/EBP β and GR are associated with the SWI/SNF complex in response to IrECM and prolactin treatment (Xu et al., 2005). In addition, this treatment promotes the binding of RNA polymerase II to the endogenous mouse β -casein promoter (Xu et al., 2005). Therefore, besides altering the nuclear localization and DNA binding properties of transcription factors, exposure to IrECM promotes transcription factor-mediated recruitment of chromatin remodeling factors to the bovine BCE-1 enhancer and the endogenous mouse β -casein promoter. A localized decondensation in chromatin structure would allow additional factors such as RNA polymerase II to access their target DNA sites and potentiate further rounds of transcription (Figure 3). Such events support a currently popular paradigm for transcriptional activation that describes the recruitment of specific transcription factors to their cognate DNA sequences and their subsequent recruitment of chromatin remodeling- and DNA-modifying enzymes followed by the general transcriptional machinery (Kosak and Groudine, 2004).

Interestingly, mouse mammary epithelial cells transiently transfected with a construct containing the BCE-1 element positioned upstream of a minimal bovine β -casein promoter cloned adjacent to a CAT reporter gene did not display an increase in BCE-1 activity upon exposure to IrECM and prolactin, while cells stably transfected with the same construct displayed a significantly

large induction (Myers et al., 1998). The fact that BCE-1 required integration into the genome to become transcriptionally active suggests that the proximity of this sequence to its target transcription factors, chromatin remodeling factors and the basal transcriptional machinery likely plays an important role in its activation. Thus, to accurately depict the factors and events involved in ECM-mediated gene expression, we must consider how the ECM could influence the general organization of the nucleus.

V. Potential Mechanisms Through Which ECM Influences the General Organization of Nuclear Factors & Overall Transcriptional Activity

The nucleus has a 10 micron diameter and contains approximately two meters of DNA (Getzenberg et al., 1991; Hager et al., 2004; Misteli, 2005). In order for this vast amount of DNA to fit within the confines of the nuclear envelope, it must be assembled into chromatin that undergoes several orders of compaction resulting in a DNA-protein assembly that differentially impedes the access of transcription factors to their cognate sites. Residing alongside chromatin is a large number of nuclear proteins involved in diverse nuclear processes such as transcription, RNA splicing and DNA repair. Add to this the presence of RNA and proteins required for maintaining structural integrity of the nucleus and it becomes clear that the nucleus is a very crowded organelle indeed.

Past studies have shown that transcription factors are located in discrete bodies within the nucleus (Handwerger and Gall, 2006; Lamond and Spector, 2003; Zimmer et al., 2004). A number of investigators have speculated that these bodies are either protein storage sites or centers for the recruitment of specific regulatory cofactors in response to extracellular and intracellular signals (Boisvert et al., 2000; Borden, 2002; Pearson and Pelicci, 2001). Factor

recruitment to nuclear bodies is important for events such as proliferation, senescence, and apoptosis. It is thus clear that understanding the factors that affect nuclear organization and the role of nuclear organization in gene expression is essential for understanding cell development and ECM-induced differentiation.

A). Of Mouse and Women: Application to Human Breast Epithelial Cells

The principles learned from the phenotypic and functional behavior of mouse mammary epithelial cells in 3D culture have been applied to human cells with an added advantage: the 3D assay provides a rapid and robust assay with which to distinguish between non-malignant and malignant breast cells (Bissell et al., 2005; Bissell et al., 1999; Petersen et al., 1992; Schmeichel and Bissell, 2003). To examine the principle of ECM signaling to the nucleus in human cells, we showed that culturing non-malignant HMT-3522 (S1) human mammary epithelial cells on lrECM gels induced acinar morphogenesis, and caused the nuclear mitotic apparatus (NuMA), and the serine/arginine repeat-related nuclear matrix protein of 160 kDa (SRm160), to coalesce into discrete foci (Knowles et al., 2006; Lelievre et al., 1998). SRm160 is a splicing factor found in interchromatin granule clusters (IGCs). IGCs are considered to be storage sites for splicing factors (Huang et al., 1994; Misteli et al., 1997; Spector, 1993). Importantly, disturbing the integrity of NuMA foci with an antibody to its C-terminal end reversed lrECM-mediated changes in chromatin organization, disrupted the BM integrity and activated one or more matrix metalloproteinases (MMPs). Treatment of cells on lrECM with trichostatin A, a histone deacetylase inhibitor, also resulted in the disruption of NuMA foci, and the loss of BM. Plachot and Lelievre (2004) subsequently showed that treatment of acini formed by S1-HMT3522 cells with 5-aza-2'deoxyctidine, an inhibitor of DNA methylation, induced DNA hypomethylation and prevented the establishment of apical polarity (Plachot and Lelievre, 2004). These results

lend further credence to the concept of a dynamically reciprocal interaction between the nucleus and the ECM, and show that DNA organization, nuclear matrix organization and the BM are all intimately connected. While the principles appear to hold true, the exact events through which ECM influences nuclear and chromatin organization remain to be resolved.

B). ECM-induced differentiation may involve the selective activation of particular tissue-specific genes, but an overall decrease in gene activity

Exposure of mouse mammary epithelial cells to lrECM decreased total levels of acetylated histones (Pujuguet et al., 2001) and the assembly of human mammary epithelial cells into functionally differentiated acinar structures on lrECM (Barcellos-Hoff et al., 1989; Petersen et al., 1992) is accompanied by a decrease in overall acetylated H4 levels, an increase in HP1 γ , and an increase in the expression levels of MeCP2 (Plachot and Lelievre, 2004). Because these and the other results cited in the previous subsection promote and/or are a consequence of gene silencing, the findings suggest strongly that lrECM-induced differentiation of human mammary epithelial cells may involve changes in chromatin structure that are conducive to selective activation of particular tissue-specific genes, but an overall decrease in gene activity.

Exposure to lrECM promotes cell cycle arrest (Fournier et al., 2006; Petersen et al., 1992) and this effect may play an important role in its ability to decrease overall transcriptional activity. For instance, in the nuclei of cells in G0/G1, the retinoblastoma (Rb) protein is complexed with *c-abl*, a nonreceptor tyrosine kinase (Welch and Wang, 1993; Welch and Wang, 1995). When associated with Rb, nuclear *c-abl* is inactive; in cycling cells, however, cyclin-dependent kinases phosphorylate Rb and disrupt this complex, causing activation of nuclear *c-abl* which, in turn, activates RNA polymerase II by phosphorylating its C-terminal repeated domain (Baskaran et

al., 1996; Baskaran et al., 1993). In a recent study, the number of active RNA polymerase II molecules and the number of active transcription sites significantly decreased in mouse F9 teratocarcinoma and totipotent mouse embryonic stem cells when they were induced to differentiate (Faro-Trindade and Cook, 2006). Thus, exposure to lrECM may decrease total levels of transcriptional activity by indirectly decreasing RNA polymerase II activity.

A decrease in the number of transcription sites would eliminate the need for certain transcription factors and create a temporary surplus of these factors that could be redistributed to discrete nuclear domains such as promyelocytic leukemia (PML) nuclear bodies for storage and/or eventual degradation (Borden, 2002; Kosak and Groudine, 2004; Zimmer et al., 2004). In support of this possibility, increasing the levels of a particular transcription factor by transient or stable expression causes excess factors to localize into PML bodies (Tsukamoto et al., 2000).

A decrease in demand for transcription factors may also reduce the need for splicing factors. The C-terminal tail of the largest subunit of RNA polymerase II recruits splicing factors from nearby IGCs during transcription (Misteli and Spector, 1999). Treatment with α -amanitin, a specific inhibitor of RNA polymerase II (Lindell et al., 1970), causes these clusters to round up and prevents the movement of splicing factors from IGCs (Misteli et al., 1997). Thus, a decrease in overall transcription levels resulting from exposure to lrECM could be responsible for the formation of SRm160 foci we observed in mammary epithelial cells (Lelievre et al., 1998). In support of this, hnRNP RNA processing proteins were shown to accumulate into HERDS, heterogeneous clusters, within the interchromatin space in response to transcriptional arrest (Biggiogera et al., 2004).

C). ECM-induced changes in overall chromatin structure may have profound implications on nuclear organization and gene expression

ECM-induced foci formation may be explained also by the ability of lrECM to promote an overall decrease in total histone acetylation levels. In proliferating monkey CV-1 cells, the formation of a nuclear body containing class I and II histone deacetylases, as well as SMRT and NCoR nuclear receptor corepressors were dependent on histone deacetylase activity (Downes et al., 2000). The integrity of these domains was disrupted by treatment with histone deacetylase inhibitors, substantiating again that the act of histone deacetylation plays an important role in nuclear organization. A function for DNA-mediated organization of nuclear factors is supported also by the findings of Kaminker and colleagues (2005) which showed that lrECM-induced cessation of growth in epithelial breast cell lines led to the formation of TIN2 foci. However, whereas treatment with DNase I eliminated the foci, treatment with RNase did not. One possible explanation for histone deacetylase-mediated formation of nuclear domains could be that histone deacetylation promotes the formation of highly condensed chromatin structures that would restrict the access of transcription factors to their target DNA sequences, thereby causing them to either localize to more accessible regions of the nucleus or amalgamate with nearby domains enriched in transcription factors. Such an event would also promote HERD formation since it would contribute to a decrease in transcription and RNA synthesis.

VI. Advancing Towards a Deeper Understanding of the Malignant Phenotype

Based on the discussion presented so far, our understanding of the effects of ECM on cellular, and, in particular, nuclear events important for normal cell differentiation is in its infancy.

Furthermore, much remains to be learned of the effect of ECM on tumor cell development. We

have shown previously that non-malignant S1 and malignant T4-2 mammary epithelial cells express similar levels of $\beta 1$ -integrin and the coxsackievirus and adenovirus receptor (CAR) when cultured on a 2D plastic surface (Anders et al., 2003; Weaver et al., 1997) (see Table 2). However, when cultured in 3D lrECM, the expression levels of these receptors decline dramatically in non-malignant cells, but remain unaltered in malignant cells (Anders et al., 2003; Weaver et al., 1997). In addition, unlike S1 cells, T4-2 cells cultured on lrECM express a profile of cell surface receptors that favors increased proliferation through pathways involving the epidermal growth factor receptor (EGFR) and phosphatidylinositol 3-kinase (PI3K) (Liu et al., 2004; Wang et al., 1998; Weaver et al., 1997).

Interestingly, the levels of $\beta 1$ -integrin, CAR, phosphorylated Akt and EGFR dramatically decline in T4-2 cells when cultured on 3D lrECM in the presence of a reverting agent that promotes the assembly of these cells into organized, polar structures closely resembling those that formed by non-malignant cells (Anders et al., 2003; Liu et al., 2004; Wang et al., 1998; Weaver et al., 1997). In addition, exposure to reverting agents induces T4-2 cells to express the tumor suppressor protein, anti-zuai-1 (AZU-1), to levels observed in S1 cells when cultured on either 2D plastic or 3D lrECM (Chen et al., 2000). In a recent study, lrECM-induced morphogenesis of normal breast epithelial cells was shown to cause a drastic change in NuMA nuclear organization without inducing morphogenesis or changes in the distribution of NuMA in malignant cells (Knowles et al., 2006; Weaver et al., 1997). Thus, the nature with which a non-malignant cell communicates with its 3D environment at both the cell surface and the nucleus differs from that of a malignant cell.

The ability of reverting agents to reverse the expression of T4-2 cell surface receptors and signaling proteins shows that the acquisition of a malignant phenotype is accompanied by changes in tissue architecture and reversible changes in protein expression that allow a transformed cell to bypass the strict hierarchical events inherent to normal cell differentiation. With this said, additional studies on normal and malignant mammary epithelial cells need to be performed to further understand and identify the cellular and nuclear events that allow a cancer cell to flourish uncontrollably in an environment that promotes the differentiation and growth cessation of normal cells.

VII. A 3D Reconstruction for the Future of Cancer Research

The union between a cell and its surrounding environment has profound implications on cellular processes such as proliferation, differentiation and apoptosis. However, during tumorigenesis, the communication between a cancer cell and its environment becomes altered. Mechanisms once enacted to provoke controlled growth and tissue-specific gene expression become obsolete, conferring a tumor cell with a growth advantage non-existent to a normal cell. While efforts have been made to elucidate the mechanisms through which ECM influences normal and tumor cell behavior and development, the underlying complexity with which a cell communicates with its external environment has thwarted advancements towards obtaining a deeper and truer understanding.

The fact that only a handful of studies have looked at the relationship between ECM and the nucleus is surprising since it is now accepted that ECM signals to gene expression and almost every cellular component outside of the nucleus is affected by ECM. One can only imagine what intriguing discoveries remain to be uncovered in the nucleus by future investigations! Such

efforts will not only provide valuable basic information about signaling regulation but also may lead to identification of novel targets for drug therapy.

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Table 1. ECM-Response Elements Identified to Date.

| Element | Gene | Type of ECM | Cell Type | Organism | References |
|-------------------|-------------------|-------------|--------------------|------------|---------------------------|
| BCE-1 Enhancer | β -casein | lrECM | Mammary epithelial | Cow | Schmidhauser et al., 1992 |
| Promoter | Erc α | Laminin | Mammary epithelial | Mouse | Novaro et al., 2004 |
| Enhancer | Albumin | Collagen | Hepatocytes | Mouse | DiPersio et al., 1991 |
| Proximal G-String | LpS1 | Collagen | Aboral Ectoderm | Sea Urchin | Seid et al., 1997 |
| Promoter | MMTV | lrECM | Mammary epithelial | Mouse | Schmidhauser et al., 1994 |
| Enhancer | SV40 ^a | lrECM | Mammary epithelial | Mouse | Schmidhauser et al., 1994 |
| Enhancer | CMV ^b | lrECM | Mammary epithelial | Mouse | Schmidhauser et al., 1994 |

^a SV40 represents Simian vacuolating virus 40.

^b CMV represents cytomegalovirus enhancer

Table 2. Expression profile of cell surface and signaling proteins in non-malignant (S1) HMT-3522 cells and malignant T4-2 cells cultured on 2D plastic and 3D hECM.

| | 2D | | 3D | | |
|---------------------------|---|---|---|--|---|
| |  |  |  |  |  |
| | S1 | T4-2 | S1 | T4-2 | T4-2R |
| EGFR | — ^b | ■ | — | ■ | — |
| β1 integrin | ■ | ■ | — | ■ | — |
| P-Akt ^a | N/A ^c | N/A | — | ■ | — |
| AZU-1 | ■ | — | ■ | — | ■ |
| CAR | ■ | ■ | — | ■ | — |

^a P-Akt represents phosphorylated Akt

^b Thin lines represent negative expression; Solid boxes represent positive expression

^c N/A represents not available

Figure 1. A schematic diagram illustrating the basic principles of dynamic reciprocity between neighboring cells and their extracellular environment. Mechanical and biochemical signals received at points of cell-cell or cell-ECM contact are transduced to the nucleus by transmembrane receptors, signaling molecules and cytoskeletal components where they initiate nuclear events resulting in the expression of specific gene products that are excreted back into the extracellular milieu. Green arrows represent the bidirectional flow of mechanical and biochemical signals between the ECM and the nucleus. RTKs represents receptor tyrosine kinase. (Modified, with permission, from Bissell et al., 2005).

Figure 2. An illustration of the different levels through which ECM controls gene expression and tissue function. As cells transition from a 2D monolayer to a 3D environment, they undergo changes in cell shape that influence the expression of certain genes. Exposure to ECM engages specific cell surface receptors and initiates the transduction of biochemical and mechanical signals through the cell to the nucleus, where they further influence gene expression. As the duration of exposure time to ECM increases, cells undergo morphogenic events involving the formation of acinar structures and once again exhibit changes in their gene expression profile. Thus, tissue structure influences gene expression and, therefore, dictates tissue function. (Modified, with permission, from Bissell et al., 2005; Bissell et al., 1999; Roskelley et al., 1995).

Figure 3. Schematic representation of lrECM- and prolactin-induced changes in nuclear organization and transcription factor binding in mammary epithelial cells that lead to the

initiation of β -casein gene transcription. In the absence of prolactin and lrECM, STAT5 is predominantly cytoplasmic in primary cells. Exposure to lrECM and prolactin induces STAT5 phosphorylation, nuclear translocation and binding to its cognate DNA sequence in the β -casein promoter. In addition, this treatment increases the association of acetylated histones and promotes the binding of additional transcription factors including C/EBP β , SWI/SNF, GR and RNA polymerase II to the β -casein promoter. Ac represents acetylation at lysine residues along histone N terminal tails.

Figure1

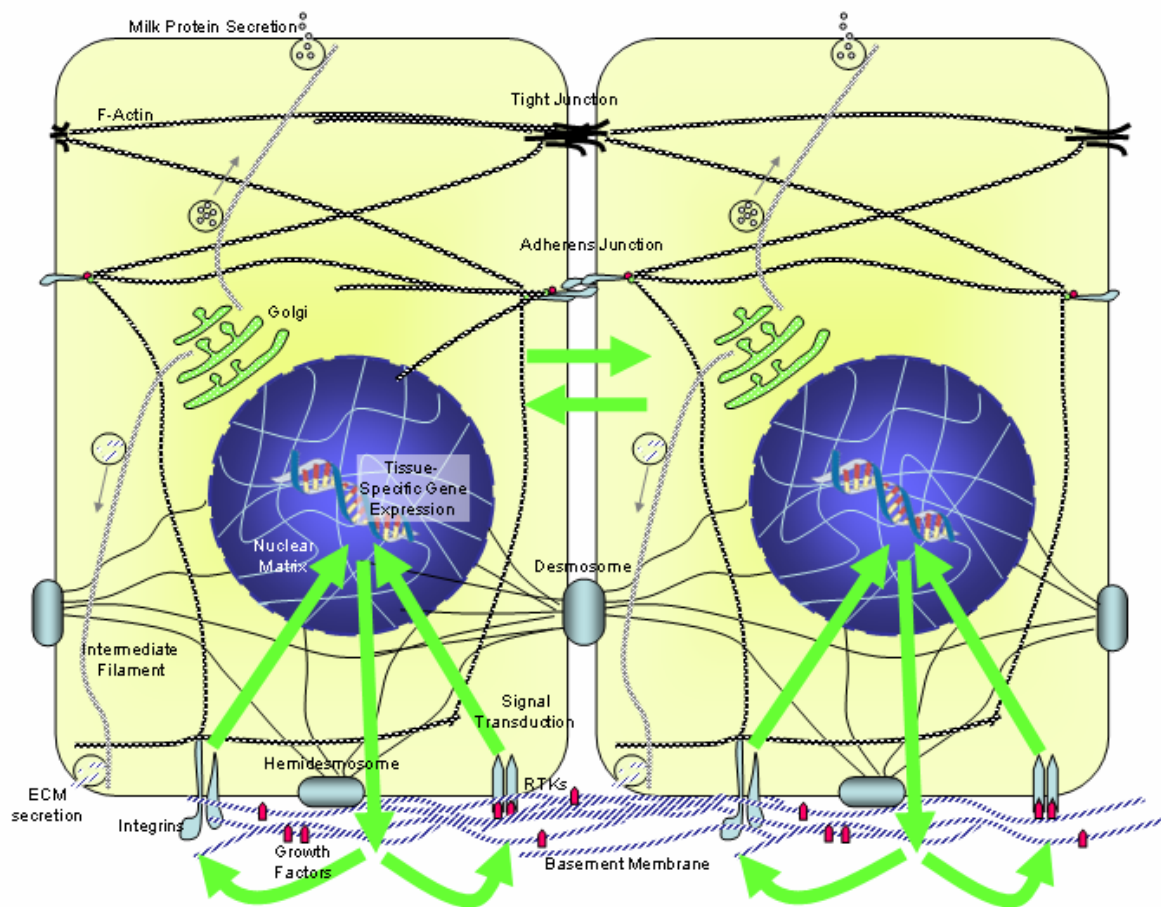


Figure2.

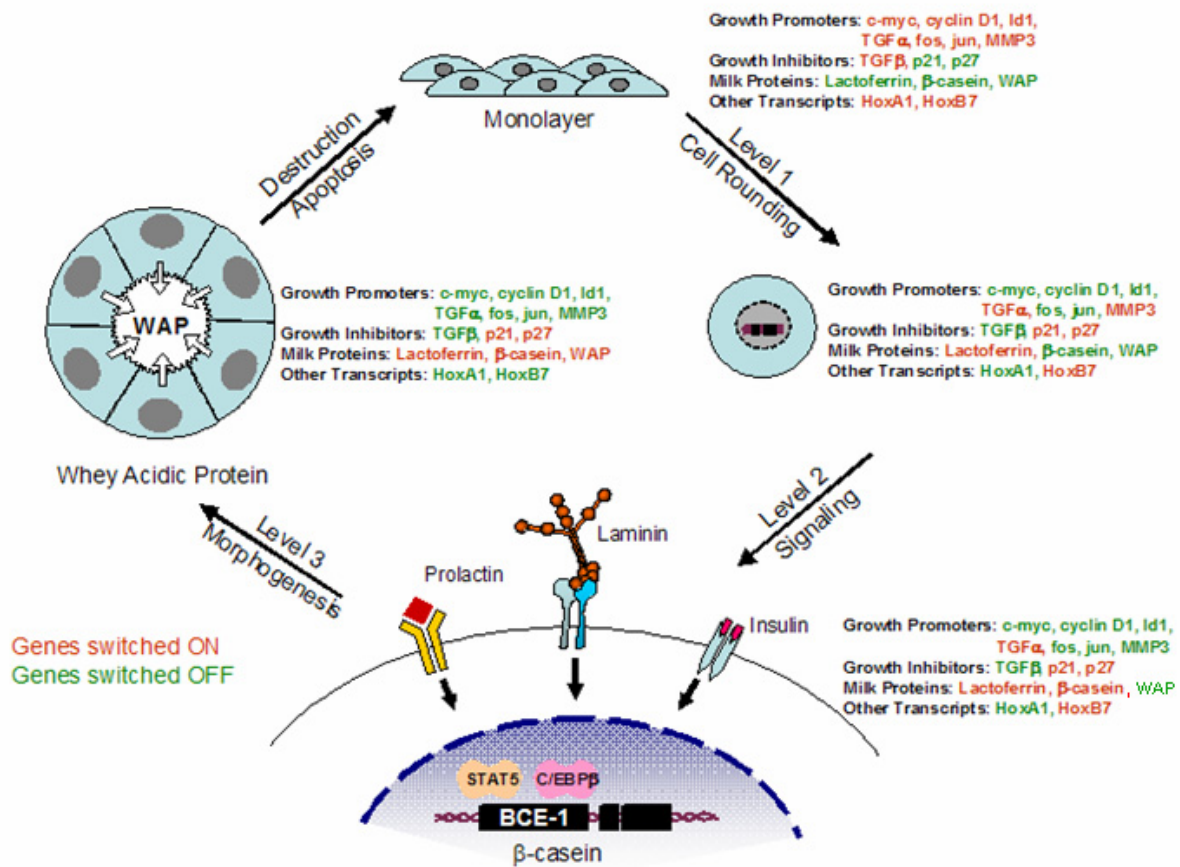
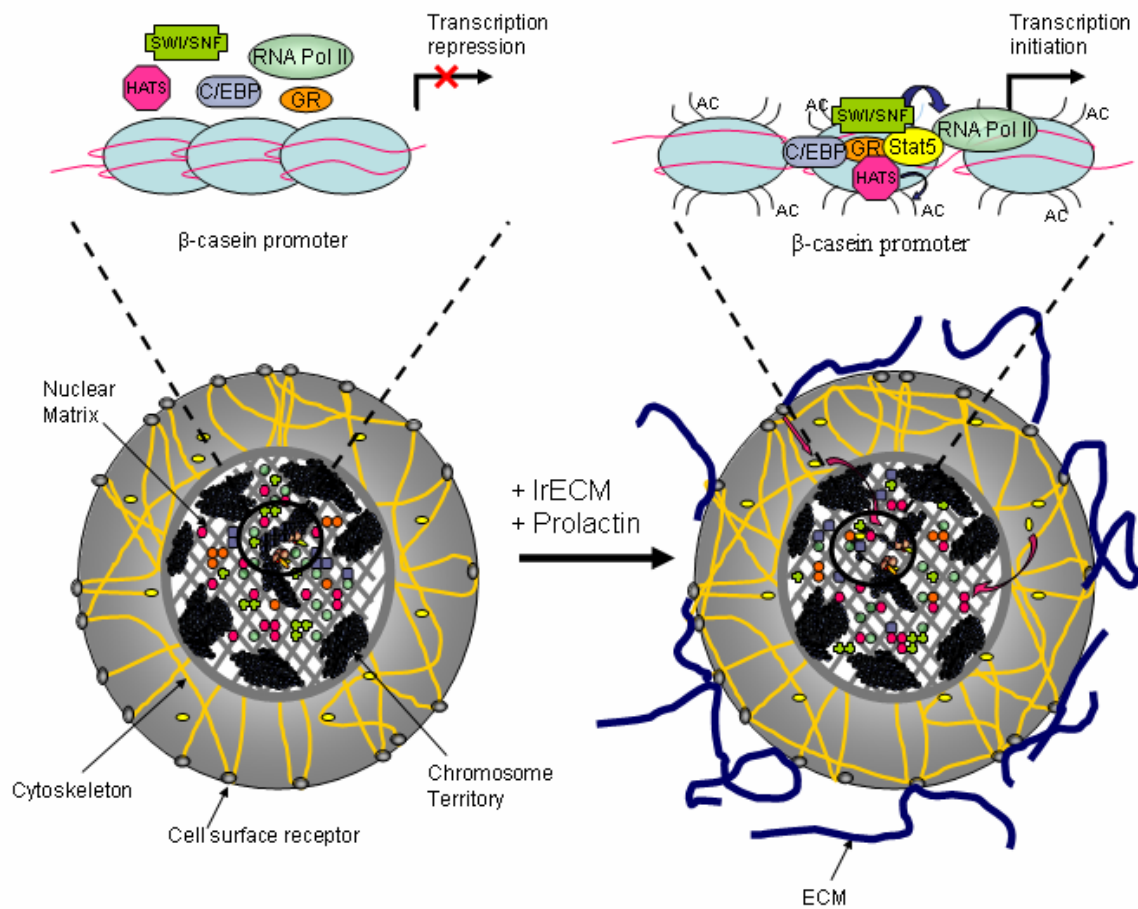


Figure3



AACR *American Association for Cancer Research*

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Chief Executive Officer

August 1, 2006

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Re: Figure 1 from Bissell, et al., "Tissue Structure, Nuclear Organization, and Gene Expression in Normal and Malignant Breast" *Cancer Research* 1999 59: 1757s-1764s.

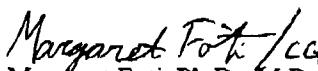
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